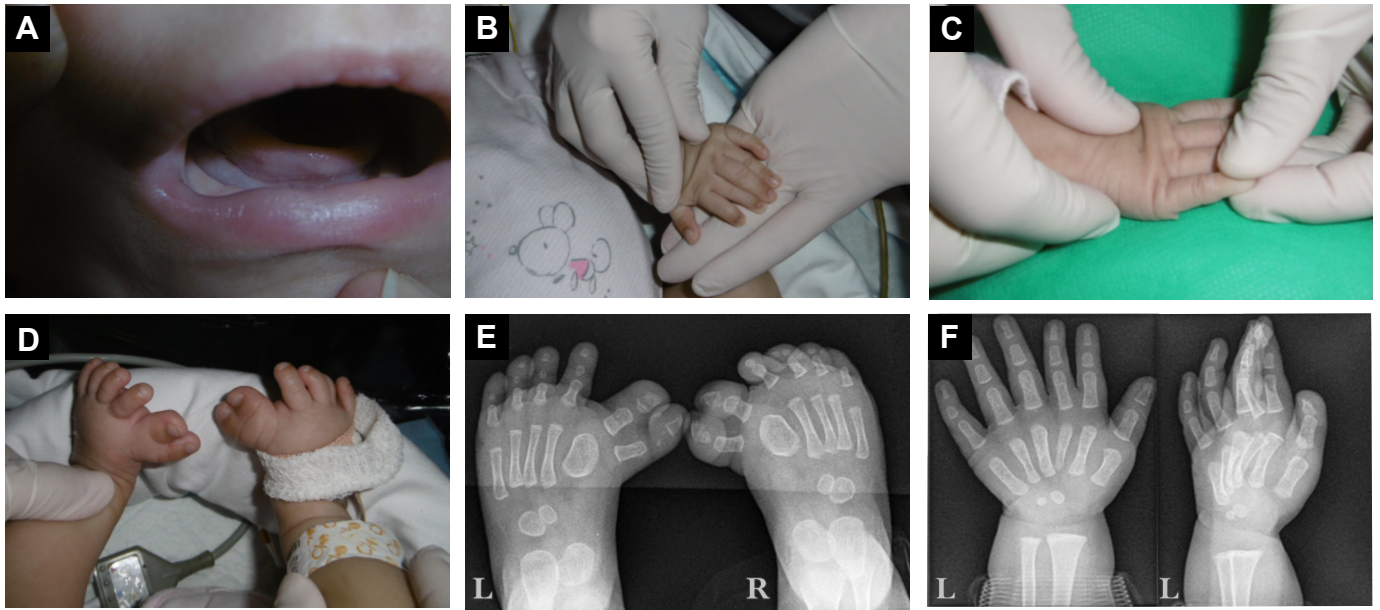
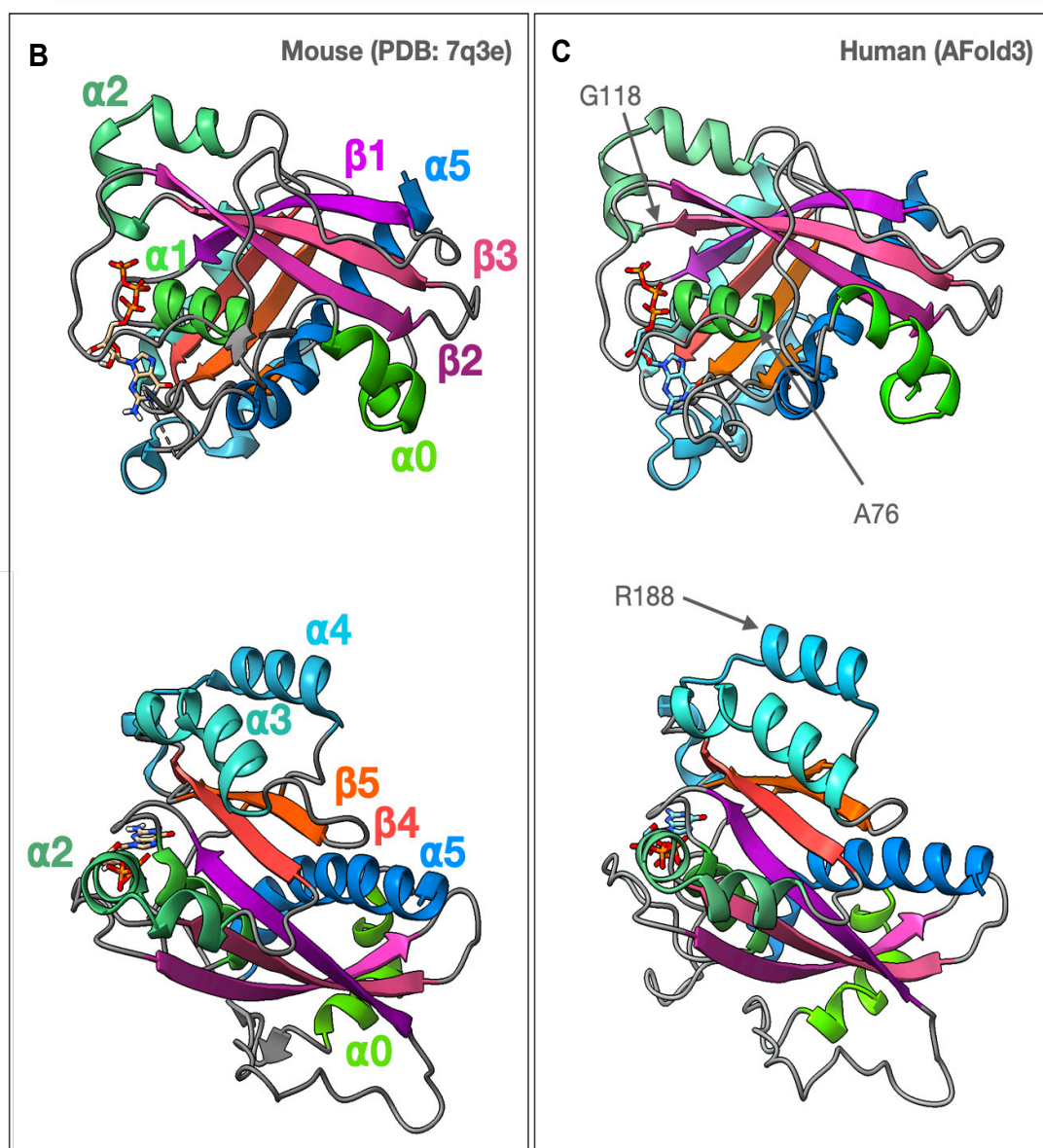
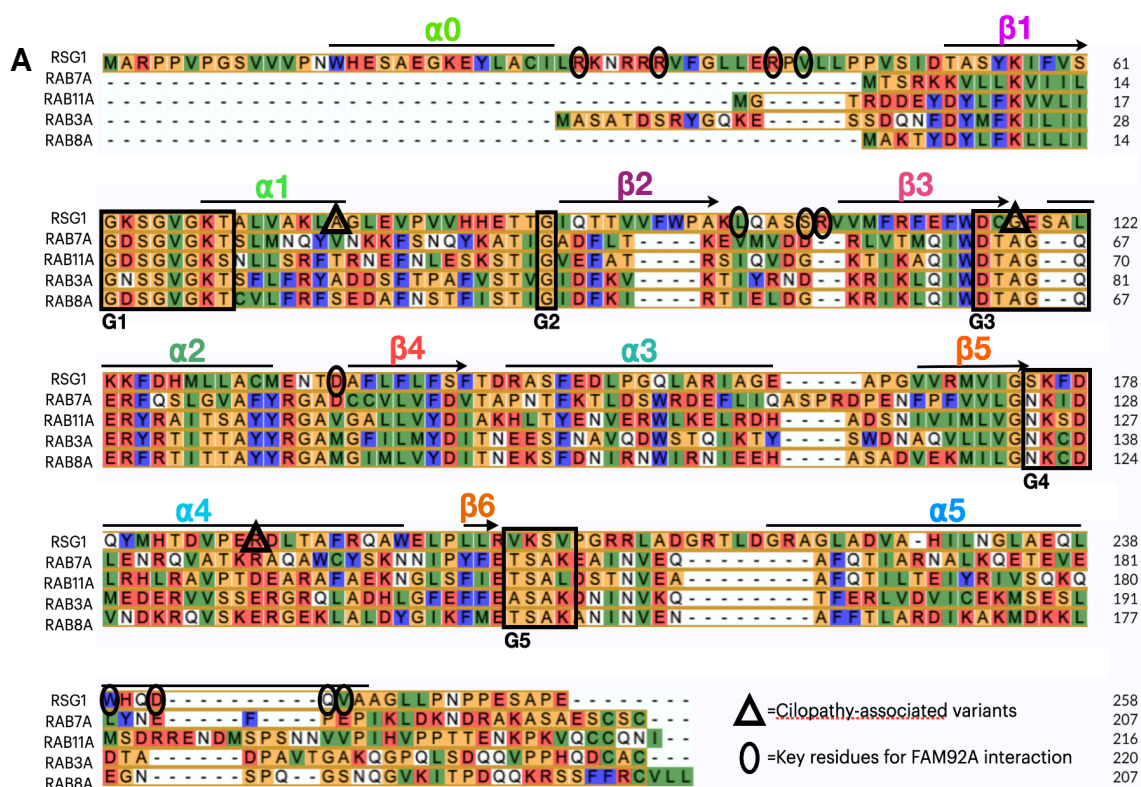
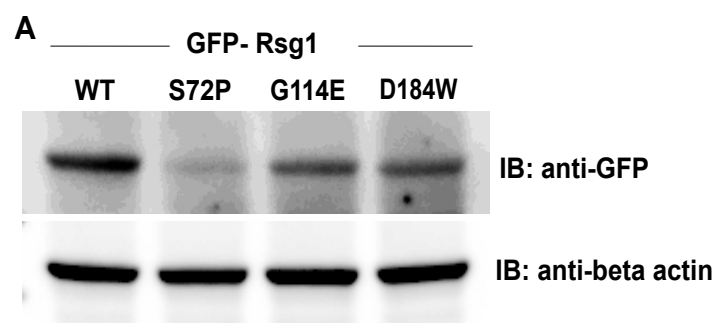
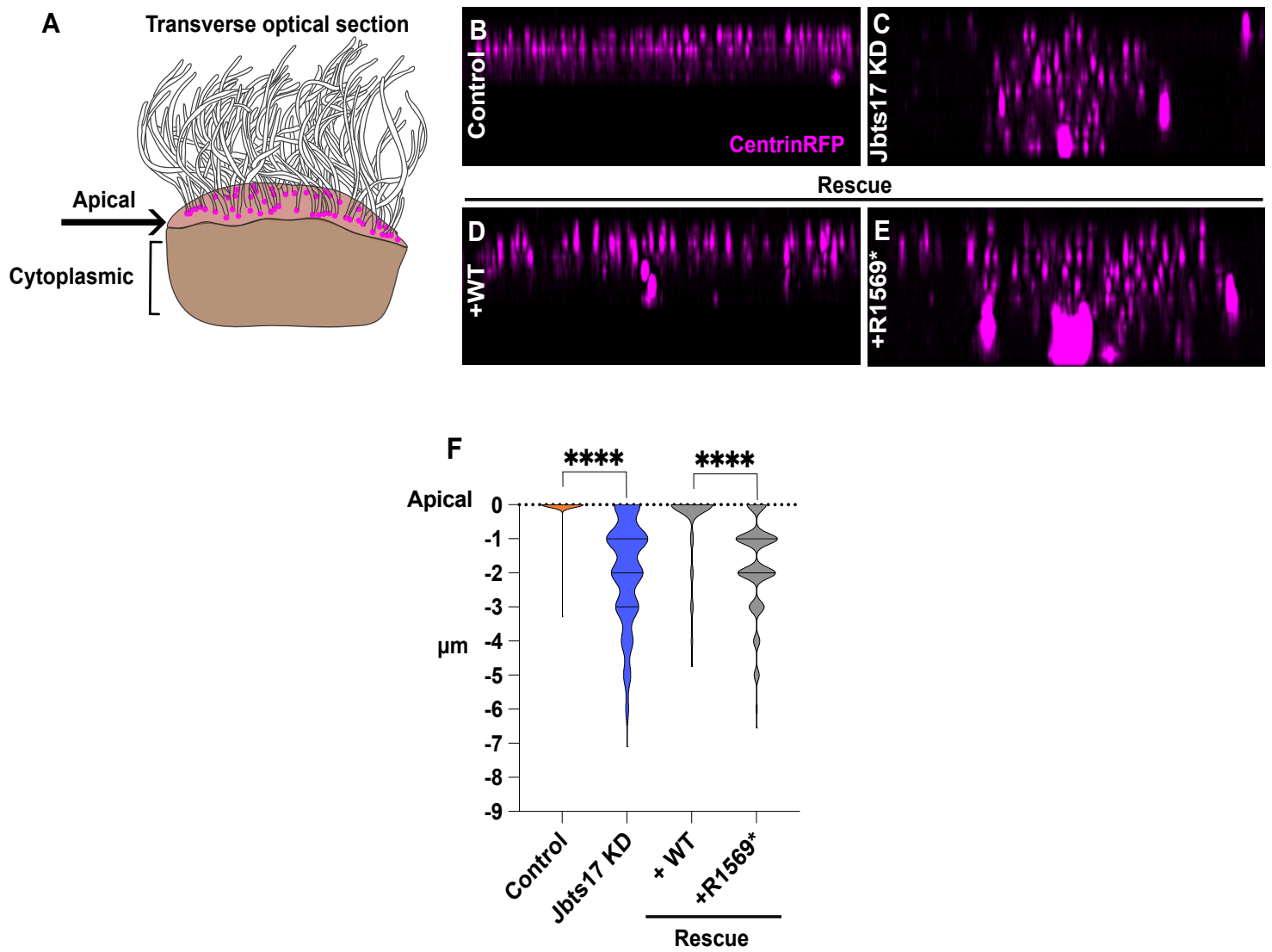
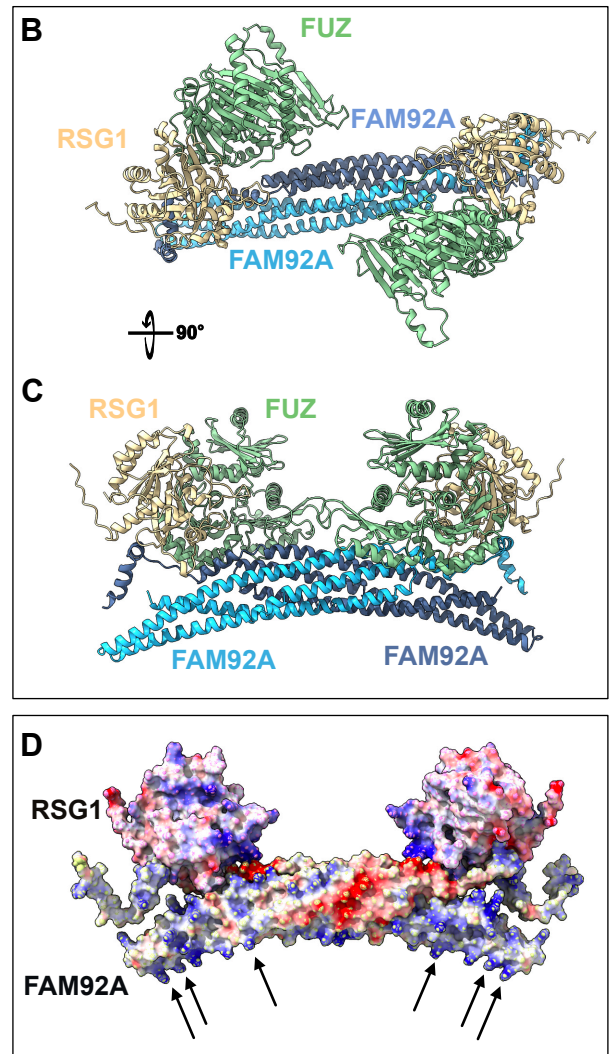
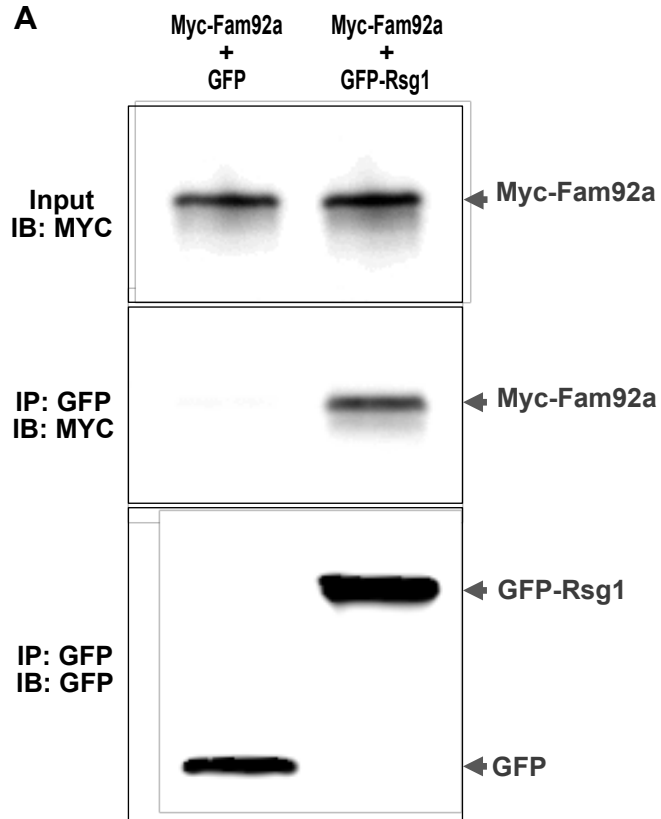


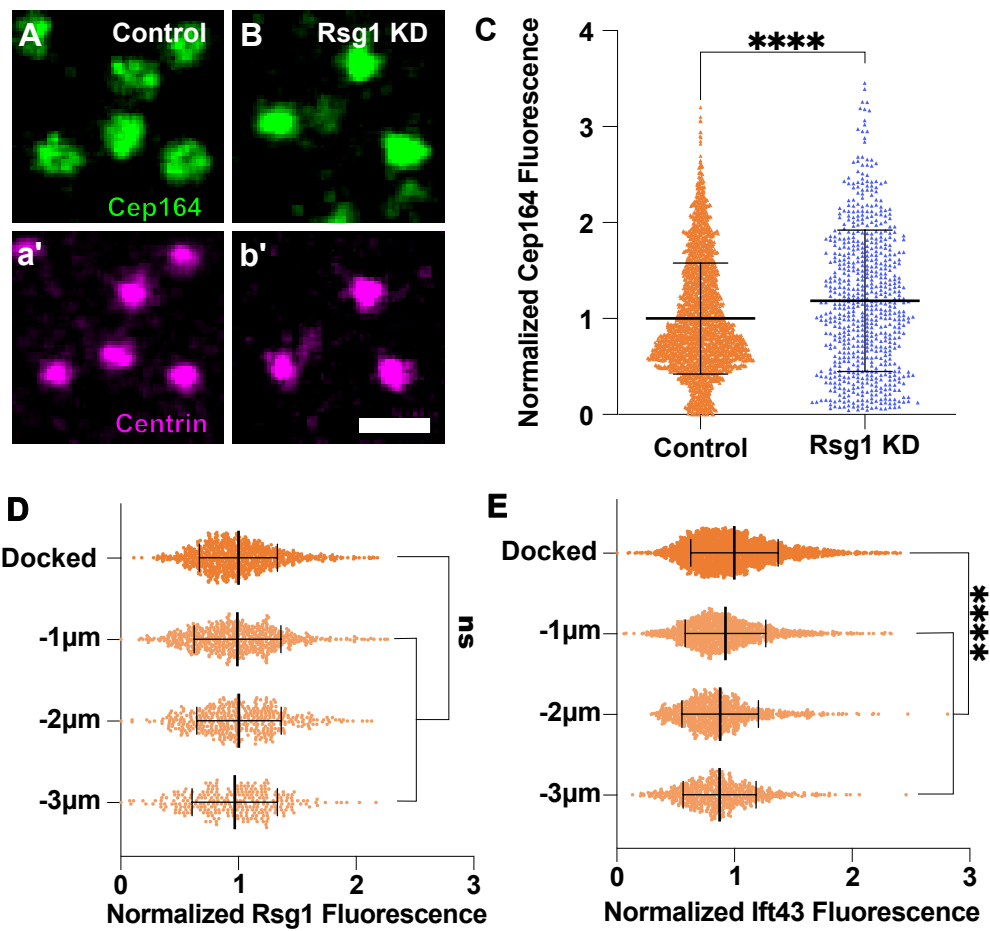


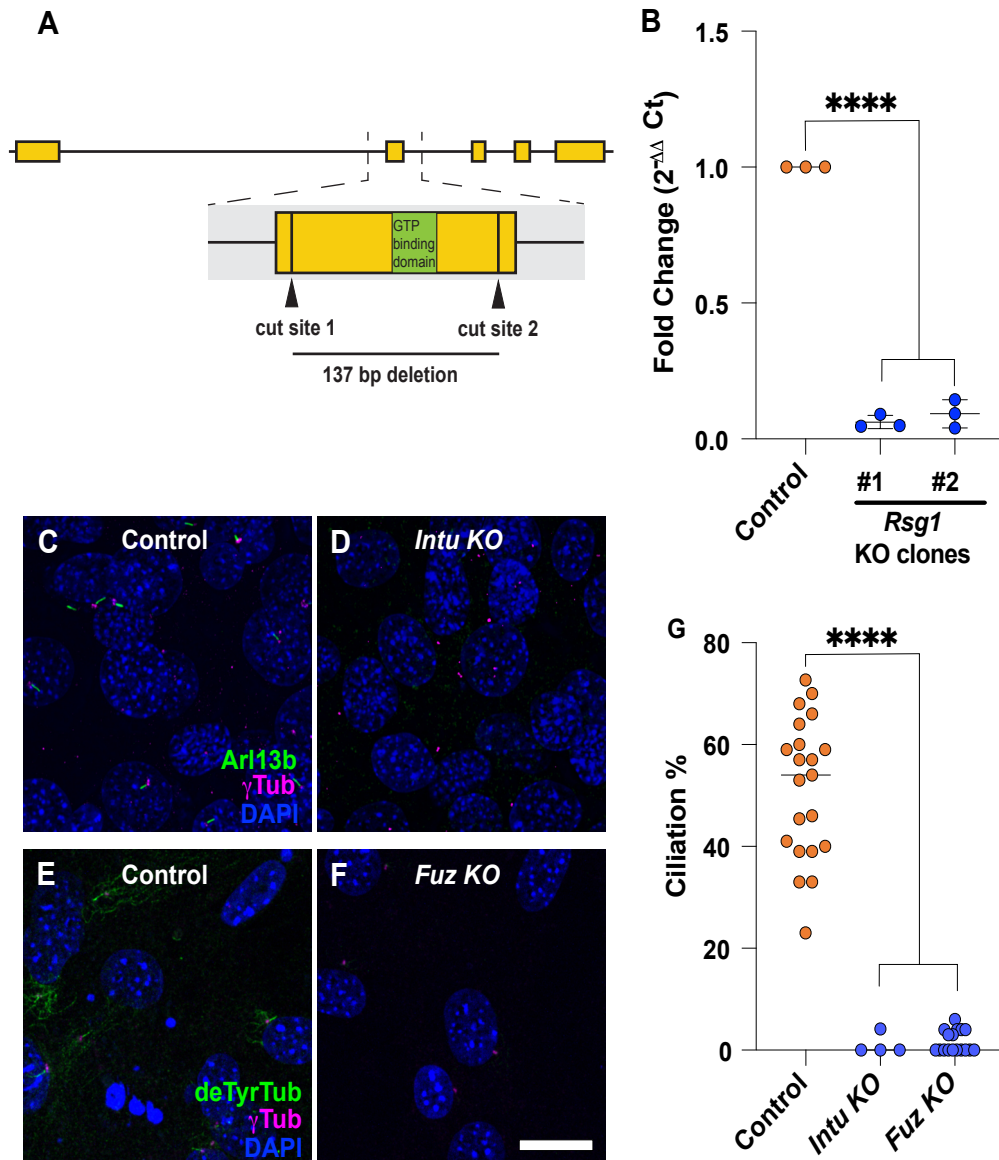












Supplemental Figure Legends:

Supp. Fig 1. Photographs and X-rays of Patient 2. A. The image shows a lobulated tongue. B and C. Show polydactyly in both hands: insertional in the left hand and postaxial in the right hand. D. Preaxial polydactyly is observed in both feet. E and F. X-rays of the same patient where polydactyly is evident in both hands and feet.

Supp. Fig 2. RSG1 sequence and protein structure. (A) Alignment of human RSG1 with related human RAB proteins. Triangles indicate ciliopathy loci identified here. Residues indicated by ovals are the top-scoring residues predicted to mediate interaction with RSG1 by computational alanine scanning using BALas (see Methods). G domains are indicated by black boxes and structural elements related to Panel B are indicated above each sequence. (B) Structure of Mouse Rsg1 (PDB: 7q3e) colored to indicate the conserved helices and sheets characteristic of small GPTases. (C) Afold3-predicted structure of Human RSG1 colored to indicate the conserved helices and sheets characteristic of small GPTases and the positions of ciliopathy loci identified here indicated in grey.

Supp. Fig 3. Alignment of vertebrate Rsg1 proteins. The L and S alloalleles of the *Xenopus laevis* allotetraploid genome are indicated together with three representative mammals. Triangles indicate ciliopathy loci identified here. Note high conservation of residues indicated by ovals, which are the top-scoring residues predicted to mediate interaction with RSG1 determined by computational alanine scanning using BALas (see Methods).

Supp. Fig 4. Protein stability of RSG1 disease alleles. (A) Western blot showing protein levels for indicated disease alleles when expressed in *Xenopus* embryos.

Supp. Fig 5. CPLANE Jbts17 disrupts basal body docking. (A) Schematic representation of a MCC, depicting the apical and cytoplasmic regions shown in the optical view. (B-E) Transverse 3D projection of centrin (magenta). (F) Graph shows the distribution of centrin signal through the cell at each μm depth starting from apical slice at 0. $N > 12$ cells for all conditions.

Supp. Fig. 6. Direct binding between Rsg1 and the c-terminus of Fam92a. (A) Myc-Fam92a, GFP-Rsg1 and GFP were in vitro translated using the TNT SP6 High-Yield Wheat Germ Protein Expression System. Myc-Fam92a was immunoprecipitated by GFP beads when co-expressed with GFP-Rsg1, but not with GFP alone, indicating a specific interaction between Rsg1 and Fam92a. (B, C) AlphaFold2 model of FUZ/RSG1/FAM92A with a configuration similar to that predicted by Afold3 (compare with Fig. 6). (D) Figure shown in C with charges of residues indicated, arrows indicate lysines and arginines required for FAM92 binding to negatively charged lipids.

Supp. Fig. 7. Distal appendage component Cep164 accumulates at basal bodies after KD of Rsg1, and Ift43 and Rsg1 localize to basal bodies before docking apically in *Xenopus* MCCs. (A, B) High magnification *en face* images of MCCs apical basal bodies labeled by centrin (magenta) and Cep164 (green) scale bar= 1 μ m. (C-E) Graphs showing mean $\pm$ standard deviation of normalized fluorescence. (C) Graph showing Cep164 basal body fluorescence after Rsg1 KD. (D-E) Graphs show the quantification of normalized fluorescence intensity of Rsg1 (D) and Ift43 (E) on basal bodies at different depths starting from apical (docked) and several μ m from the apical surface. N> 23 cells in 5 embryos across 2 experiments for all conditions. N values and statistics can be found in Supp. Table 3.

Supp. Fig 8. Generation and validation of RPE1 *RSG1* knockout cell lines and validation of Fuz and Intu mouse embryo fibroblasts. (A) CRISPR-Cas9 mediated *RSG1* knockouts were generated in RPE1 cells using two synthesized sgRNAs designed to cut on either side of the GTP binding domain within exon 2 of *RSG1*. Two monoclonal cell lines were isolated containing the same 137 base pair deletion. (B) RT-qPCR delta delta Ct analysis indicates a significant reduction in *RSG1* gene expression in both isolated knockout clones compared to a non-targeting control line. N=3 biological replicates, error bars are represented as mean with SD; **two-tailed P value $\leq$ 0.01 and ***two-tailed P value $\leq$ 0.001 (one-sample t test, theoretical mean = 1). (C-F) Immunofluorescence of mouse embryonic fibroblasts of indicated genotype with DAPI (blue) and gamma tubulin (magenta). Ciliation % was quantified using deTyrosinated Tubulin(green) in Fuz and Arl13B (green) in Intu. Scale bar = 20 μ m. (G) Percentage of deTyrTub (green) and ARL13B (green) positive cells in *Fuz* and *Intu* mutants. N> 80. N values and statistics can be found in Supp. Table 3.

Supplemental Table legends:

Supp. Table 1: Spreadsheet of clinical data for patients.

Supp. Table 2: Spreadsheet of molecular characteristics of patient alleles.

Supp. Table 3: Statistics and N values for all experiments.

Supp. Table 4: Spreadsheet of proteomic data for APMS of WT Rsg1 and Rsg1(T69N). Full are available in the PRIDE database (Project accession: PXD055830; Project DOI: 10.6019/PXD055830).